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ON THE SUBMAXILLARY GLAND OF THE DOG
RECORDED WITH CONTINUOUS PHOTOGRAPHIC METHODS

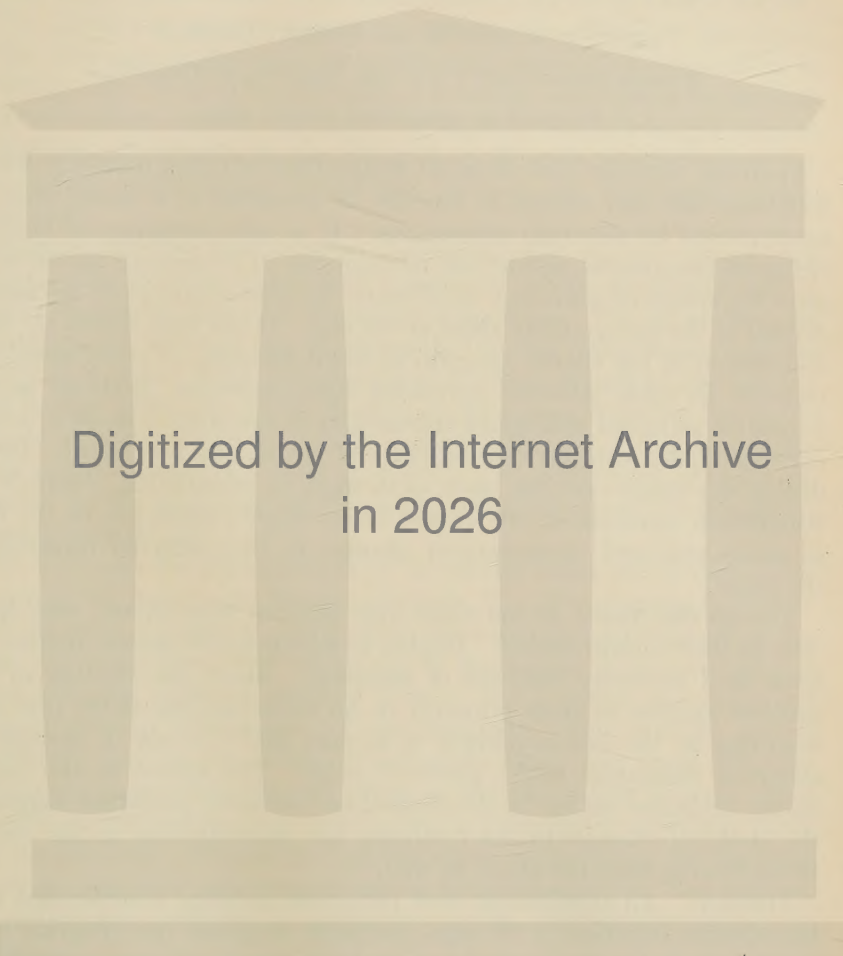
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ELECTRICAL CONDUCTIVITY, ELECTRICAL POTENTIAL AND HYDROGEN ION CONCENTRATION MEASUREMENTS ON THE SUBMAXILLARY GLAND OF THE DOG RECORDED WITH CONTINUOUS PHOTOGRAPHIC METHODS

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From the structure and chemical composition of living matter it seems inevitable that any change in function or condition of a tissue must be accompanied by electrical phenomena. It is only necessary to observe stringent precautions against the introduction of extraneous variables to show the effects of numerous variables on the characteristics of the action current of the submaxillary gland of the dog. It has been shown (1) that stimulation of the chorda tympani of equal strength, of equal duration, repeated at equal intervals provoking equal secretion, produced superimposable electrical deflections approaching in similarity a series of action currents of the regularly beating heart of the normal individual. Introducing any single variable, such as strength of stimulation, duration of stimulation, duration of rest, altered blood supply, resistance to the flow of saliva produced characteristic changes in the electrical behavior of the gland.

Though the results on the whole were uniform they offered very little clew to their interpretation. Hoping to advance this subject further we have used accessory methods of approach. Since the solution of the problem appears to hinge primarily on an understanding of the processes occurring at the cell membrane it seemed that a study of changes in electrical resistance¹ might prove of value. The effects of the highly mobile hydrogen ion on the development of electrical potentials suggested the study of changes in the hydrogen ion concentration of the venous blood flowing from the gland as well.

METHOD. All experiments were performed on dogs, anesthetized with hypodermic injection of 10 mgm. morphine sulphate per kilogram body weight followed in 20 minutes by a rectal injection of 0.8 gram urethane per kilogram body weight. The gland was dissected free of outlying fascia to

¹ Confusion frequently arises as a result of employing the term "conductivity" for both electrical and physiological conductivity. It therefore seems desirable to use "electrical resistance" when referring to the former quantity and to reserve "conductivity" for use when referring to the transmission of a physiological disturbance.

permit direct application of electrodes—thus minimizing electrical resistance in circuit. The chorda lingual and vago-sympathetic nerves were exposed and precautions for uniform stimulation observed (1). A glass cannula was inserted high in Wharton's duct. All branches of the external jugular vein with the exception of those draining the gland were ligated to permit measurements of the volume-flow of blood from the external jugular vein.

The gland was usually excited by tetanic stimulation of its nerves. In the early part of the work the stimulating current was furnished by an induction coil. Due to the difficulty of correctly ascertaining the strength of the secondary current, and the annoying induced disturbances inherent in this form of stimulus, it was replaced by 60 cycle alternating current. The reduced stimulating voltage was read on the alternating current voltmeter.

Salivary secretion was recorded photographically by the opaque drop method. The secretion was led into a system of two displacement bottles—the first containing mineral oil, the second opaque ink. The saliva displaced the mineral oil which in turn displaced the ink, this falling from a tube immersed in a vessel of oil immediately in front of the slit of the camera. This method slows the falling shadow of the drop sufficiently for photographic registration and by greatly increasing the size of the drop it produces a legible record of a rapid secretion. The volume-flow of blood was recorded by the same method, the blood dropping directly in a separate vessel of mineral oil.

The hydrogen ion concentration of the venous blood was recorded with the use of the manganese dioxide electrode (2). The changes in E.M.F. resulting from changes in acidity were photographed with the use of the string galvanometer. The advantage of employing a vacuum tube in circuit with the electrode occurred to us in that it draws no current and therefore avoids polarization of the electrode. This method proved satisfactory but in the animal experiments there was some difficulty encountered in avoiding induced effects of the stimulating current. This led us to use the simpler method of connecting the string directly in series with the electrode. Due to the high resistance of the string, the fact that the string was fully compensated before every observation, and that we were interested primarily in qualitative results, the method served our purpose.

Zinc-zinc sulphate non-polarizable electrodes used early in the research for recording electrical potential and electrical resistance were soon replaced by La Picque electrodes of disc design, 5 mm. $\times$ 5 mm. square. The discs were wrapped in a thin layer of absorbent cotton and sewed in place on the gland. This procedure insures a constant position of the electrode with respect to the gland and permits closure of the wound, thus preventing disturbances through changes in temperature and drying.

The electrical deflections were recorded by means of a d'Arsonval galvanometer² operated potentiometrically by the gland through a vacuum tube type UV 201. When that surface of the gland to which the grid was connected became positive with respect to the other surface which was connected to the filament, the plate current increased and vice versa.

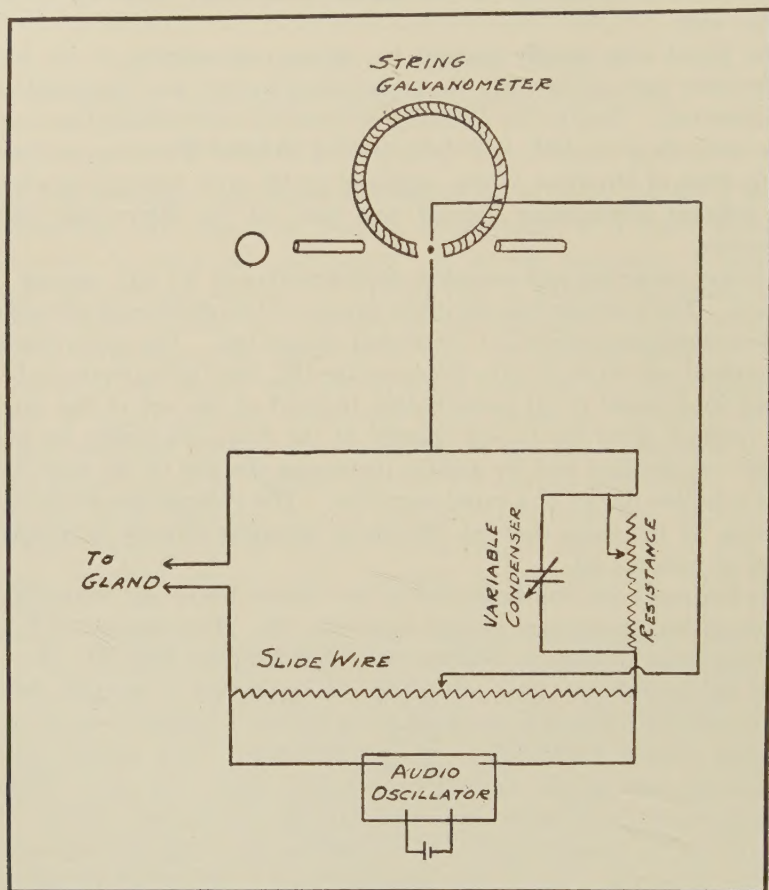


Fig. 1. String galvanometer as resistance recorder

There was, accordingly, a fluctuation of current through, and a variation of potential across, a 150,000 ohm resistance in the plate circuit. Inasmuch as the galvanometer was connected across the ends of the resistance it was this magnified potential variation that caused the deflection. A potenti-

² This galvanometer had a sensitivity of 13,000 megohms (7.7×10^{-11} amperes), a period of 14 seconds, resistance of 570 ohms, and a critical damping resistance of 22,000 ohms. It was at a distance of 105 cm. from the camera.

ometer in series with the latter made it possible to balance the constant plate current and thus keep the galvanometer compensated. In addition to amplification of the electrical deflections the vacuum tube offered the distinct advantage of the use of the same electrodes for recording both changes in electrical potential and electrical resistance.

So far as we are aware, no suitable method has been employed for recording changes in electrical resistance. The simplest and most direct method of substituting a string galvanometer for the telephone in the classical bridge method was tried, figure 1. A change in resistance registers as a change in width of the shadow of the vibrating string. This method—particularly if the unbalance were amplified—should prove valuable but the advantages of the second method will be apparent.

This consists of an alternating current bridge, a three-stage thermionic amplifier, and a direct current Wheatstone bridge. The three are so combined that a change in conductivity of the tissue under study alters the balance of the alternating current bridge and consequently the input voltage to the amplifier. This variation of input voltage changes the conductance of the last tube, the plate to filament circuit of which is one arm of the direct current bridge. The result is a deflection of the direct current recording galvanometer placed in this second bridge circuit.

Figure 2 gives the details of the circuit. The gland, or other tissue, is placed in one arm of the alternating current bridge; a variable resistance, *A*, and complementary portions of a slide wire resistance, *C*, comprise the remaining three arms. The source of alternating current is a 1000 cycle oscillator, *B*, of the commercial type. The telephone usually employed for detecting the bridge balance is replaced by the primary of an audio frequency transformer, *D*, the secondary being across the grid and filament of a type UV199, or other suitable thermionic tube. The grid of this tube is maintained at the proper potential by means of the battery, *F*, and the potentiometer, *G*. The plate circuit of this first tube consists of a 150,000 ohm resistance, *H*, and a 90 volt storage battery which also furnishes the plate voltage for the other two tubes. One end of the high resistance, *H*, is connected to the grid of the second tube through a 0.01 microfarad condenser, *I*; the other side to the filament. The 5 megohm resistance, *J*, affords a high resistance escape for the electronic charge which tends to accumulate on the grid. This second tube is coupled to the third by means of an audio frequency transformer, the grid of the third tube being isolated by a 0.01 microfarad condenser shunted by a 5 megohm resistance. The plate to filament circuit of this last tube is, as has been stated, one of the four arms of the direct current bridge; the other three are the 25 ohm fixed resistance, *K*, the 25 ohm variable resistance, *L*, and the 12,000 ohm fixed resistance, *O*. This bridge contains as the recording element a d'Arsonval galvanometer with a sensitivity of 1 megohm and a period of

less than a second. A beam of light is reflected from the galvanometer mirror onto the slit of a moving film camera so that a continuous photographic record is obtained of resistance variations. When records of very rapid changes in resistance were desired a string galvanometer was used in place of the d'Arsonval for recording purposes.

The theory of operation of the system is this: When the alternating current bridge is perfectly balanced none of the 1000 cycle current flows through the primary of the first transformer. The amplifier is consequently in equilibrium and, if the direct current bridge is balanced by the variable resistance, L , the galvanometer will be at its rest position. If, now, the conductivity of the gland changes, the alternating current bridge becomes unbalanced and a certain amount of the 1000 cycle current flows through the primary of the transformer. This causes the potential of the

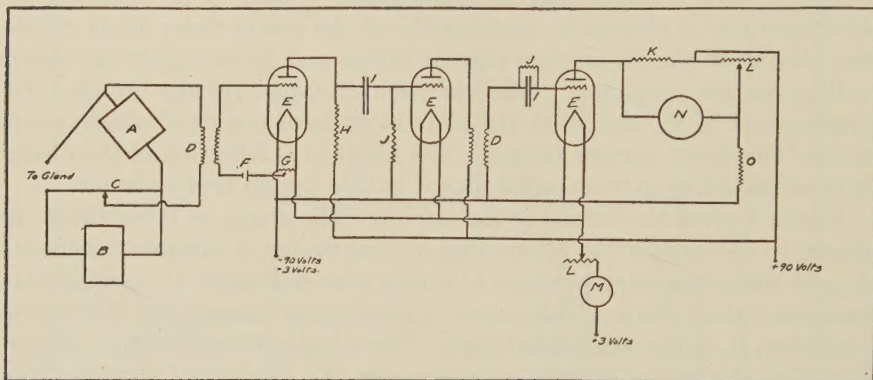


Fig. 2. Vacuum tube resistance recorder. A—variable resistance; B—audio oscillator; C—slide wire resistance; D—audio frequency transformer; E—vacuum tube; F—grid battery; G—potentiometer; H—150,000 ohm resistance; I—0.01 M.F. condenser; J—5 megohm resistance; K—25 ohm resistance; L—25 ohm variable resistance; M—ammeter; N—galvanometer; O—12,000 ohm resistance.

grid of the first tube to oscillate about its normal value, producing consequent variations of the same frequency in the plate current. Due to this alternating current through the 150,000 ohm resistance, there is an alternating voltage applied to the grid with respect to filament of the second tube of a larger value than that applied to the first. This voltage, in turn, causes the current through the primary of the second transformer to oscillate at 1000 cycles and so induces an electromotive force in the secondary very much larger than that originally derived from the alternating current bridge. During the positive half of each cycle of this amplified voltage an increased number of electrons are drawn to the grid of the third tube and, due to the action of the grid condenser, this charge is not entirely lost during the negative half of the cycle. The result is an increased

negativity of the grid relative to the filament and a consequent decrease in the plate to filament conductance. The direct current bridge is therefore unbalanced and the galvanometer deflected.

It is evident that either an increase or decrease in tissue resistance causes an increase in 1000 cycle current through the in-put transformer and a deflection of the galvanometer that is in the same direction in both cases. The following procedure was therefore employed. The reading of the galvanometer was noted for a balanced alternating current bridge; the bridge was then unbalanced by varying the ratio arms a known amount and in such a way that an increase in gland resistance caused the galvanometer to return toward its balance position. In this way the sign of the resistance variation was indicated by the direction of the galvanometer swing and the magnitude of the change was easily calculated. The following example illustrates the method. The bridge was balanced and the resistance of the gland was found to be 180 ohms. The bridge was then unbalanced to such an extent as would correspond to an increase in gland resistance of 25 ohms; the corresponding galvanometer deflection was 9.5 cm. On stimulation of the gland the galvanometer swung back 8.5 cm. toward its balance position, an increase in resistance of $8.5/9.5 \times 25$ ohms = 22.4 ohms or $22.4/180 = 12.4$ per cent.

The above computation is made on the assumption that there is a linear relationship between resistance change and galvanometer deflection. Our results show that such is practically the case provided the resistance does not increase sufficiently to bring the bridge almost into balance.

This system has a number of desirable features by virtue of which it should serve as an instrument for opening up numerous fields of physiological investigation. It gives a continuous record of changes in resistance that makes it possible to follow photographically the variations in permeability of a tissue or organ. The duration of resistance change to which it will respond is limited mainly by the rapidity of the recording instrument; in conjunction with a string galvanometer it will therefore follow exceedingly rapid fluctuations. By making the amplification factor large it will have a high sensitivity and will therefore be suitable for precise work. The circuit is such that it avoids the slow drift inherent in many amplifying systems inasmuch as the transformer coupling between the second and third tubes passes only the high frequency variations.

RESULTS. The results reported in this paper have been taken from experiments performed on twenty-eight animals and in many of the experiments a number of tests were made of each point investigated.

Certain general results of activity of the submaxillary gland may be pointed out before going on to the more detailed experiments. We have confirmed many of the previous observations regarding electrical deflections (1), (3), (4), (5). Among these may be mentioned the invariable

presence of a negative deflection³ when there is secretion produced by other than stimulation of the cervical sympathetic fibres and a positive deflection in the latter case.

Excitation of the gland sufficient to produce secretion, whether by electrical stimulation of either the cranial or cervical sympathetic nerves or by the injection of pilocarpin, caused an increase in the resistance of the gland. This increase followed the beginning of stimulation by about three seconds and frequently preceded visible secretion by several seconds or more. It generally long outlasted the period of stimulation and secretion.

Secretory activity always caused an ultimate increase in the acidity of the blood flowing from the gland. This increase in the acidity of the blood generally reached a maximum after secretion was nearly over, certainly after the height of glandular activity was passed. The exact time at which this effect was produced in the blood cannot be stated, however, due to the difficulty in determining the time that elapses between the instant the blood leaves the gland and its arrival at the electrode vessel. In certain cases

Fig. 3. Occlusion of Wharton's duct. *S*—secretion in drops; *ST*.—stimulation of chorda tympani by 1 volt A.C.; *E.R.*—electrical resistance; *E.D.*—electrical deflection (negative components upwards); *T*—time in 15 seconds; 1—occlusion of Wharton's duct; 2—deocclusion of duct; 3—occlusion of duct.

STIMULUS	RESISTANCE INCREASE IN PER CENT	E.D. IN MILLIVOLTS	pH (ARBITRARY UNITS)	SECRETION
1—Deoccluded.....	12.4	-0.192	-10	21
2—Occluded.....	16.0	-0.218	11;-18	
3—Deoccluded.....	12.8	-0.312	3;-9	28
4—Deoccluded.....	12.8	-0.182	2;-9	21
5—Occluded.....	14.6	-0.234	10;-18	

Fig. 4. Occlusion of carotid artery. *S*—secretion in drops; *ST*.—stimulation of chorda tympani by 1 volt A.C.; *E.R.*—electrical resistance; *T*—time in 15 seconds; 1—occlusion of carotid artery; 2—deocclusion of artery; 3—occlusion of artery.

STIMULUS	RESISTANCE INCREASE IN PER CENT	pH (ARBITRARY UNITS)	SECRETION
1—Deoccluded.....	11.2	-12	10
2—Occluded.....	14.7	-49	16
3—Deoccluded.....	12.0	-19	15
4—Occluded.....	15.2	-41	15

³ A negative deflection indicates the outer surface of the gland to be electrically negative to the hilus surface and is represented as an upward movement of the curves, Gesell (1). This was the lead employed for resistance measurements as well as electrical deflection.

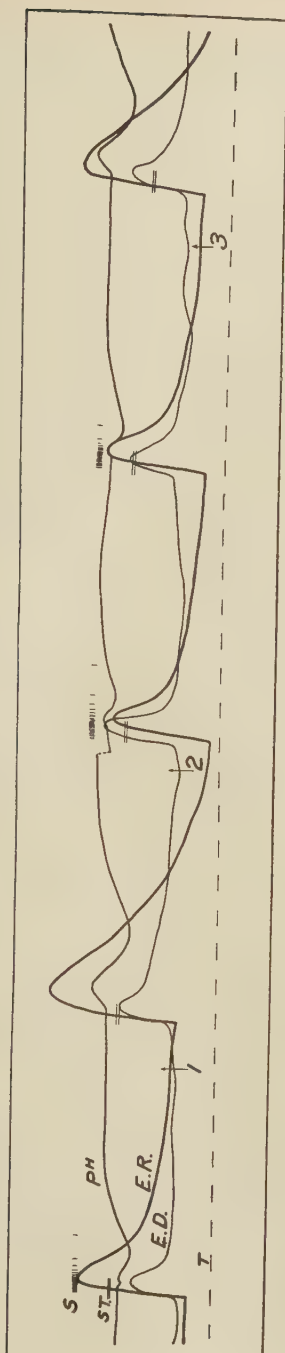


Fig. 3

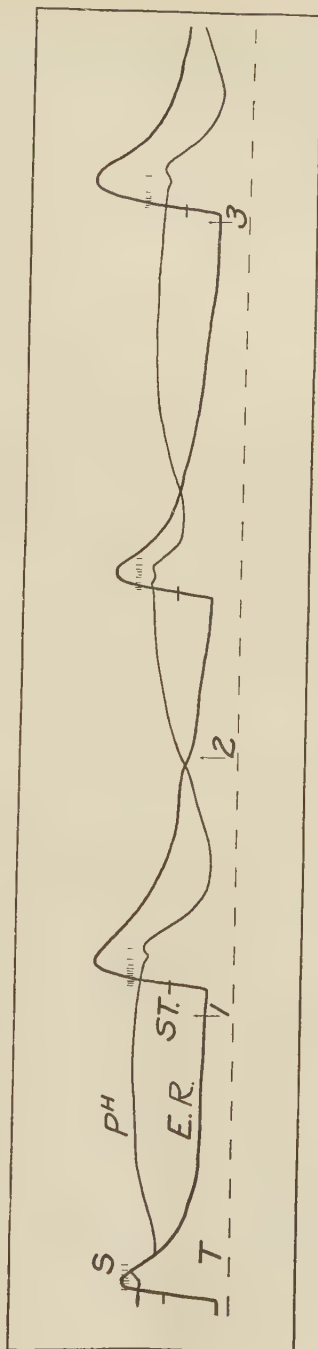


Fig. 4

there were components present in this variation other than the simple acid movement. The most prominent of these was a rather large decrease in acidity that came during, or shortly after, stimulation.

Occlusion of Wharton's duct. In studying the mechanism of secretion it seemed desirable to investigate the effect of secretion against pressure. This was done by occluding Wharton's duct. Stimulation of the chorda tympani with the duct closed is referred to as an occluded stimulus and with the duct open as a deoccluded stimulus. Characteristic results are given in figure 3 and the accompanying table.

The occluded stimuli invariably caused a greater resistance increase than the deoccluded stimuli. If the occluded stimulus was strong enough and of sufficient duration, the resistance subsequently dropped to below its pre-stimulus value. Such an after effect never resulted from stimulation with the duct open. The drop in resistance as a result of stimulation with the duct occluded was often associated with a decreased response of the gland to the following deoccluded stimulus. This was especially evident toward the end of a series and was frequently apparent in the decreased magnitude of secretion as well as resistance variation.

In eight out of ten experiments the principal component of the electrical deflection (-2) was larger as a result of a stimulus with the duct occluded than with it open. Figure 3 shows one of the two exceptions.

In every experiment the occluded stimulus produced the greater increase in acidity of the blood. Usually the alkaline component was also larger as is evident in the figure. The blood reached its maximum acidity later with occlusion and the return to the pre-stimulus value was less prompt. In a series of stimuli the change in acidity frequently became progressively greater; the electrical deflection smaller.

If there was an escape of dammed-up saliva following deocclusion there was a rapid drop in resistance of a few per cent and sometimes a small negative electrical deflection.

Occlusion of the carotid artery. In these experiments we followed the results of changes in blood supply to the gland resulting from clamping the common carotid artery during stimulation. The stimuli are also referred to as occluded and deoccluded stimuli. A record of such a series is shown in figure 4.

The resistance increased more as a result of an occluded stimulus and the rate of recovery was much slower. These results were obtained invariably although it was necessary that the artery remain occluded in order that the recovery be retarded. Similarly, the increase in acidity of the venous blood was always much larger as the result of an occluded stimulus; the maximum acidity was reached later and the return to pre-stimulus level was less rapid.

A statement as to the effect of reduced blood supply on the magnitude and form of the electrical deflections is rather precarious. In five out of eight series the magnitude of the principal component was larger as the result of an occluded stimulus but in the remaining three the results were inconclusive.

Injection of pilocarpin. This method of stimulation was particularly useful when studying the effects of prolonged secretion, as illustrated in figure 5, A and B. The injection of pilocarpin caused a sustained secretion of saliva sometimes lasting as long as half an hour. This was accompanied by a prolonged increase in resistance. The resistance increase began from five to eighty seconds before there was visible secretion but so far we have not noted an increase in resistance without an ultimate secretion. Generally, if the resistance increased very rapidly it was followed within five to ten seconds by a rapid secretion; if, on the other hand, the resistance increased but slowly, the secretion did not commence until after a comparatively long interval, and was then rather scanty. These points are admirably shown by a comparison of figure 5, A and B. In A the resistance increase was rapid and the secretion which followed within a few seconds was copious. The opposite was true as a result of the first injection in B. In this case the rather slow secretion did not begin until after the resistance had already come to constancy at the increased level.

Pilocarpin secretion was associated with a small negative electrical deflection and an increase in the acidity of the venous blood.

If the duct was occluded during pilocarpin secretion there was a marked fall in resistance and the blood became more alkaline. On deocclusion these effects were reversed, as is shown in the figure. Occlusion of the artery caused a slowing of secretion and tended to increase the acidity of the blood. Stimulation of the vago-sympathetic fibres produced an initial, very slight increase in the rate of secretion that was followed by a prolonged and considerable decrease. The accompanying resistance increase long outlasted the temporarily accelerated secretion.

A paralyzing injection of atropin stopped secretion and was accompanied by a gradual decrease in resistance and subsequent stimulation of the chorda tympani produced no variation in resistance, blood acidity, or electrical potential regardless of the strength of stimulation. Such a stimulus without response is shown in figure 5 B.

Effects of asphyxia. Seven experiments were done on the effects of asphyxia in the hope that they would throw some light on the relationship between the acidity of the blood and glandular activity. Under the conditions of these experiments increased acidity of the blood had no effect upon either secretion or upon the electrical deflections. The resistance of the gland, however, did undergo a small but definite decrease during asphyxia.

This effect is shown in figure 6. The record was taken with the gland in the resting condition and shows the characteristically slow fall and rise of resistance accompanying asphyxia and the recovery. Because of the great importance of any general relationship between blood acidity and cell permeability this phase of the investigation is being extended to other tissues.

Stimulation of the vago-sympathetic fibres. Electrical stimulation of the cervical sympathetic fibres in the vagus produced a small increase in the resistance of the gland. The increase and recovery were, however, very much slower than those resulting from chorda tympani stimulation. Even very strong stimulation failed to produce the larger changes in resistance that accompanied stimulation of the chorda tympani. The scanty secretion and positive electrical deflection reported by other investigators have been observed.

Figure 7 gives a comparison of the change in resistance resulting from stimulation of the vago-sympathetics and the chorda tympani. Although the former stimulus was longer and twice as strong the resistance increase was very much less. Figure 8 shows the effects of vagal stimulation on acidity as well. Note the greatly delayed and slow increase in resistance and acidity as compared with the prompt and rapid effects that were always produced by similar stimuli applied to the chorda tympani.

DISCUSSION. *Relation of resistance changes to electrical deflections.* In planning this work it had seemed reasonable to assume that the resistance changes of the gland would help to explain the electrical deflections because of the obvious dependence of bioelectric potentials on cell per-

Fig. 5. Injection of pilocarpin. *S*—secretion in drops; *E.R.*—electrical resistance; *E.D.*—electrical deflection (negative components upwards); *T*—time in 15 seconds.

	RESISTANCE INCREASE IN PER CENT	E.D. IN MILLIVOLTS	pH (ARBITRARY UNITS)
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Fig. 5 A

1— $\frac{1}{8}$ grain pilocarpin.....	8.4	−0.037; 0.032	−40
2—Occlusion of duct.....	−1.5	−0.035; 0.035	12
3—Deocclusion of duct.....	2.8	−0.030	−13
4—Occlusion of artery.....	−2.1	0.032	−17

Fig. 5 B

1 and 2— $\frac{1}{8}$ grain pilocarpin.....	9.5	−0.010	−11
3—Occlusion of duct.....	−6.0	−0.030	11
4—Deocclusion of duct.....	6.0	0.030	−11
5—Atropin.....	Decrease		Decrease

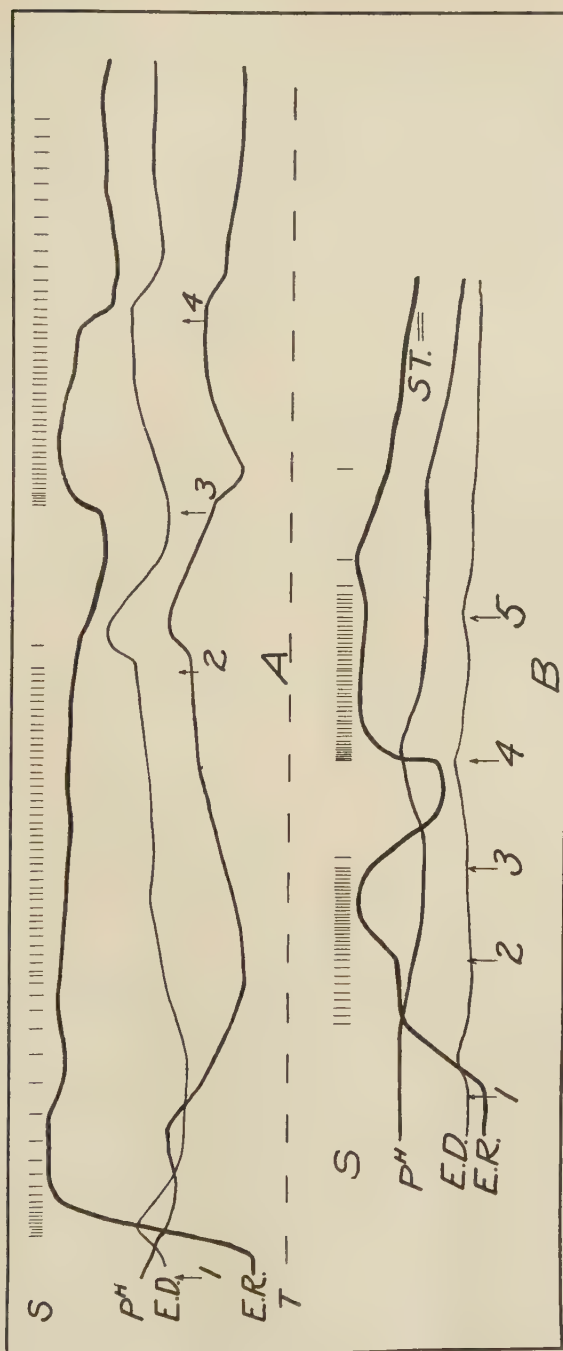


Fig. 5

meability. One of the most striking points of our work was the constancy of behavior of the gland with respect to resistance changes as compared with the variability of the electrical deflections under like conditions. A constancy of electrical deflections was obtained only by observing the most stringent precautions.

Undoubtedly one of the reasons for the greater difficulty in controlling the form of the electrical deflections is that they are the algebraic sum, the resistance changes the arithmetic sum, of the contributions from the several cells. The consequences of this difference may be brought out by

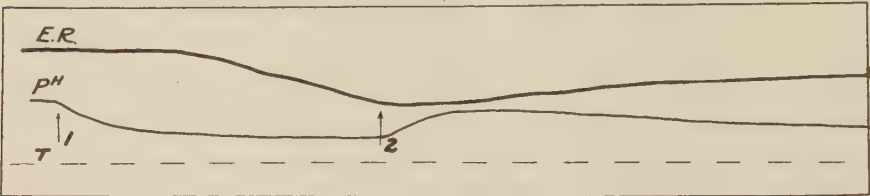


Fig. 6. Asphyxia. *E.R.*—electrical resistance; *T*—time in 15 seconds

	RESISTANCE CHANGE	pH (ARBITRARY UNITS)
1—10 per cent CO ₂	3.2 per cent decrease	−20
2—Room air.....	3.0 per cent increase	+15

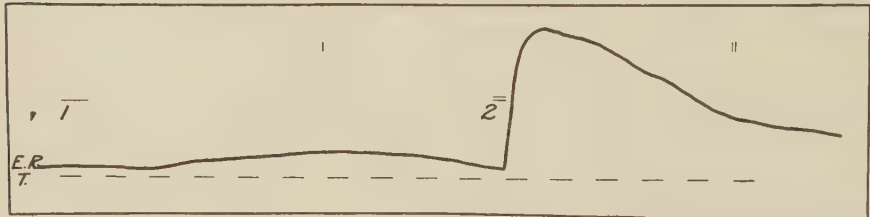


Fig. 7. Stimulation of vago-sympathetics and chorda tympani. *E.R.*—electrical resistance; *T*—time in 15 seconds; 1—stimulation of vago-sympathetics; 2—stimulation of chorda tympani with weaker stimulus than in 1.

a consideration of figure 9 which is a simple diagrammatic section through two different acini. The arrows represent the magnitude and sign of the contributions made by two of the cells to the change in potential difference between the leading-off electrodes resulting from stimulation. It is evident that the electrical deflection given by acinus A will be different from that given by acinus B, the sign of the deflection being reversed. It therefore follows that the electrical deflection given by a gland must be a complex function of the position of the leads, the magnitude of the part played by the various cells and their orientation, and the like. But the

resistance across these two acini involves only the magnitude, not the sign, of the various cellular contributions. If then the lengths of the lines represent the effective changes in resistance of the cells, the net resistance change of the two acini between the electrodes will be the same. In general, the resistance of the gland will be a very much simpler function of the position of the leads and the arrangement and condition of the individual cells. Consider, for instance, the effects of gland cells behaving as we know the component fibres of muscles do, certain cells resting while others carry on their active function. For the purpose of illustration let us make the simple assumption that all of the cells are arranged as in acinus A or B. If now eighty per cent of the secreting cells are arranged

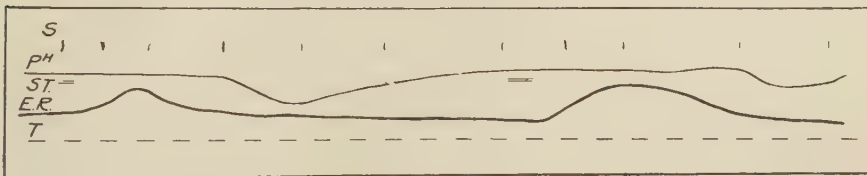


Fig. 8. Stimulation of vago-sympathetics. *S*—secretion in drops; *ST*—stimulation of vago-sympathetics with 10 volts A.C.; *ER*—electrical resistance; *T*—time in 15 seconds.

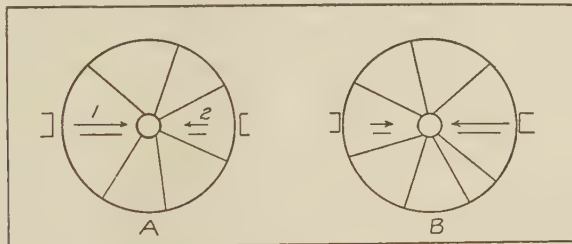


Fig. 9

as in acinus B, the potential difference will be directed from right to left; if at another instant sixty per cent are as in B and the other forty as in A, the electrical deflection will still be in the same direction but much smaller due to the balancing of oppositely directed potential gradients; while if at another time twenty per cent are as in B and eighty as in A, the size of the deflection will again be increased but will now be directed from left to right. The resistance change on the other hand will be the same in each case.

If the all-or-none-law holds for gland cells it undoubtedly contributes to the difficulty of controlling the electrical deflections. If a stimulus is delivered which is below the threshold for cell 1 but above that for cell 2, the potential difference variation will be directed from right to left,

whereas with a stronger stimulus the potential difference will act from left to right. An increasing stimulus would thus alter the sign and form of the electrical deflection but would have no effect on the resistance changes other than a greater increase in net resistance. Due to the very large number of cells which enter into the response of the gland there is no doubt but that extreme irregularities in the electrical deflections would tend to be ironed out by the averaging effect. Nevertheless there is still a possibility that such causes may account for the differences in form of response given under slightly different conditions.

Significance of the resistance changes. Before attempting any interpretation of the observed increase in resistance it is to be emphasized that vasomotor effects are not the determining factors. That the resistance changes are due to the activity of the gland cells themselves rather than to changes in the circulation is shown by the fact that stimulation of either the chorda tympani or the cervical sympathetics causes this increase in resistance in spite of the fact that the former stimulus produces an increase in the volume-flow of blood and the latter a decrease. Furthermore, there is neither resistance change nor secretion when the chorda tympani is stimulated subsequent to atropinization, although the volume-flow of blood is increased.

The results of certain investigators led us to expect a decrease in the resistance of the gland during secretion. Garmus (6), for instance, has found that the cells of the nictitating membrane gland of the frog are more than normally permeable to dyes when secreting under the influence of pilocarpin. Crozier (7) observed that during stimulation of the indicator containing tissue of the nudibranch *Chromodoris* a slimy secretion was expelled from the surface, and the rate of penetration of dichloroacetic acid was greatly increased. And Lillie (8) states that "although the factors are complex, there seems to be little doubt that the transport of secretion across the cell boundary is associated with an increased permeability to water as well as to dissolved substances."

The difference between this generally accepted conception and our results may be accounted for by the fact that two differently functioning membranes are involved in the secretory process—those facing the lumen and those toward the basement membrane and adjacent cells. Thus it is possible that stimulation causes an increase in permeability on the lumen side but a decrease on the side toward the basement membrane. Inasmuch as the cell membrane toward the lumen has the smaller area (due to the wedge-shaped arrangement of the cells around the tubules and ductules) the result will be the observed increase in net resistance. If, in addition, there is a breakdown of intra-cellular substance into a larger number of osmotically active particles, water will be drawn in from the lymph and will carry with it the dissolved substances into the duct.

The two differently functioning membranes thus explain the properly directed secretory current as well as the resistance increase.

This conception of a differential change in membrane permeability may also be used to explain the passage of water from the osmotically more active blood into the osmotically less active saliva on the basis of electro-endosmose. Bernstein (9) among others has shown that if two solutions are separated by a semi-permeable membrane, it is possible for the water to flow from the more concentrated to the less concentrated against the osmotic pressure gradient. Due to the greater permeability of the membrane to certain ions than to others there is a difference in their concentrations on the two sides and consequently a potential difference between the two. And so the water molecules having acquired a positive charge by selective adsorption, or by contact, are caused to migrate from the positive to the negative side of the membrane. With a properly directed potential gradient the movement will therefore be against the osmotic pressure.

To explain the mechanism of resistance increase we might assume that in the resting condition the side of the cell toward the basement membrane is more or less permeable to both anions and cations with a consequently small or zero potential difference between the opposite surfaces of the cell membrane. If on stimulation the permeability to the anions within the cell decreases, there will be an increase in net resistance and a potential difference between the two sides of the membrane. Due to this potential gradient water will be drawn in from the lymph spaces and thus produce the secretion.

There are, of course, other possible factors which may play a contributory part at least in the secretory process and thus influence the resistance. It is, for instance, conceivable that the resistance variations are a function of cellular and intra-cellular acidity. It is well known that the conductivity of a protein salt solution depends on the acidity and is a minimum at the iso-electric point. Likewise, Osterhout (10) shows that the permeability of protoplasmic membranes varies with the concentration of H ions. With these facts in mind we set out to study the effects of mechanical asphyxia and administration of CO₂ on the response of the gland to the usual stimuli. The resulting increase in blood acidity threw no light on the problem, however, for the only observed effect of the increased blood acidity was a decrease in the resistance of the gland whereas glandular activity always caused both an increase in blood acidity and an increase in resistance.

Admitting that the complete answer to the problem may include other considerations, we will for the present hold to the tentative assumption that the one side of the cell decreases in permeability while the others increase or remain constant and that the secretion is effected by the

drawing through of water by osmosis or electroendosmose or by a combination of the two. The evidence in favor of the resistance variation being due to such a reversible change in membrane permeability is strengthened by the behavior of the gland during a series of stimuli with the duct alternately occluded and deoccluded. Figure 3 shows that after a prolonged stimulation with the duct occluded the resistance falls to below its pre-stimulus value. It is quite probable that this decrease is due to the injury suffered by certain of the cells in secreting against pressure for Osterhout (11) has found that an invariable consequence of cellular injury is a decrease in resistance. Furthermore, the usual result of decreased resistance resulting from injury is a decreased irritability. In this connection we have repeatedly found that when the gland is caused to secrete into an occluded duct, so that the resistance subsequently falls to below its pre-stimulus level, the next stimulus with the duct deoccluded produces less than the usual response,—the resistance increase, electrical deflection, and secretion all being smaller.

Influence of blood supply on resistance changes. If then the increase in resistance is due to a reversible change in the permeability of the membranes it seems reasonable to assume that the recovery of normal resistance is dependent upon an adequate blood supply. Stimulation of the chorda tympani for instance produces a rapid increase in resistance followed by a slower return to normal. Barcroft's determinations show that there is at the same time an increase in the oxygen consumption of the gland of from three to four-fold and an increased volume-flow of blood (12). If the recovery of normal resistance is dependent on blood supply, then stimuli which are accompanied by a smaller than normal increase in blood-flow should produce greater than normal increases in resistance because the change in the membranes would be able to proceed further before being effectively checked by the recovery process. This is shown to be the case by the unusually large increase in resistance resulting from stimuli with the duct or artery occluded, for in both instances the volume-flow of blood increases less than is usual on stimulation of the chorda tympani. The contrast between a temporary and a protracted deficiency in blood supply is likewise illustrated by these two types of stimuli. Following stimulation with the duct occluded the volume-flow of blood is greatly accelerated for some time, but after stimulation with the artery occluded there is no such increase. In the former case the resistance promptly recovers its normal value, while in the latter the recovery is usually much delayed. Here again resistance recovery seems to be conditioned by the blood supply.

The behavior of the gland as a result of stimulating the vago-sympathetic fibres during pilocarpin secretion gives another instance of the correspondence between reduced blood flow and increased resistance. The volume-flow of blood decreases and the resistance increases. This may

be accounted for as follows. During the time that the pilocarpin is effective in producing secretion it tends to keep the resistance at a higher level although this is constantly opposed by the recovery process. A state of equilibrium is attained resulting in a temporary constancy of resistance. When the volume-flow of blood is reduced by stimulation of the vago-sympathetics, the action of the pilocarpin in maintaining a higher level of resistance becomes more effective, the equilibrium is destroyed, and the resistance increases until a new equilibrium level is reached.

Stimulation of the vago-sympathetic fibres. It might be argued that if the result of a smaller blood supply is a greater increase in resistance, stimulation of the vago-sympathetics should produce a greater increase in resistance than a like stimulation of the chorda tympani. The reverse actually happens. The reason may lie in the fact that the two processes which we have suggested as combining to determine the size of the resistance change are better balanced from the start. The initial changes in permeability are probably much smaller than those resulting from chorda-tympani stimulation, as is evidenced by the smaller secretion; and the reduced blood supply is therefore adequate to hold the resistance rise to a low level. The effect of the reduced blood-flow is evident in the very much slower return of the resistance to its pre-stimulus value.

Increase in blood acidity. Our finding that the ever-present accompaniment of secretion is an increase in the acidity of the blood from the gland confirms Barcroft's (12) observation that the oxygen consumption and carbon dioxide output of the secreting gland are increased by a factor of three or four.

The greater increase in acidity that results from stimulation of the chorda tympani with the duct or artery occluded than from stimuli delivered when the duct or artery is not occluded is undoubtedly due to two effects of the smaller volume-flow of blood in the former cases. A given response of the gland results in the passage of a certain quantity of metabolites from the cells to the blood. A decreased blood-flow would therefore cause a greater concentration of acid metabolites and a correspondingly higher acidity. And, furthermore, the anaerobic conditions would probably cause a greater lactic acid formation. The increase in acidity when the carotid artery is occluded during pilocarpin secretion is also to be accounted for on the basis of the above two factors.

It is doubtful, however, if the differences in volume-flow of blood resulting from stimuli with the duct open or closed are sufficient completely to account for the greater blood acidity in the latter case. A plausible way of explaining the difference is to assume that the gland does more work in secreting against pressure and consequently has a higher rate of metabolism. Inasmuch as the blood-flow does not increase sufficiently to meet this greater demand for oxygen the result must be an increased acidity.

SUMMARY

Electrical resistance, electrical deflections and acidity of the venous blood of the submaxillary gland of the dog were photographically recorded.

A vacuum tube method to follow rapid changes in electrical resistance was developed, and the methods recently devised for following changes in acidity of the circulating blood were adapted to photographic registration with and without the use of the vacuum tube. The electrical deflections were recorded with the d'Arsonval galvanometer in conjunction with a vacuum tube.

The necessity of providing uniform conditions for the elicitation of uniform electrical deflection on stimulation of the chorda tympani was confirmed.

In marked contrast to the variability of the electrical deflections obtained under less stringent conditions was the uniformity of directional changes in electrical resistance.

Provided visible secretion was elicited an increase in electrical resistance invariably occurred.

The difference in behavior of the gland in these respects is probably accounted for by the fact that the electrical deflection is an algebraic sum of the potential differences of individual cells, whereas the net resistance is the arithmetic sum of the resistances of the several cells.

It is suggested that the changes in resistance may be accounted for by a constancy or decrease in resistance of the lumen side of the cell and an increase in resistance of the opposite side.

Stimulation of the cervical sympathetic fibres caused a smaller and less rapid increase in resistance than stimulation of the chorda tympani or the injection of pilocarpin.

With the duct occluded during stimulation of the chorda tympani the resistance increase was greater than normal, but subsequently fell below its pre-stimulation value. This may be due to cellular injury.

Retarded blood supply during stimulation likewise elicited a greater increase in resistance followed by a slower return to normal. This may be explained by the dependence of cell permeability and of recovery on blood supply—a retarded blood-flow permitting the resistance to reach a higher level before the establishment of equilibrium with the recovery processes.

Secretory activity of the gland was accompanied by increased acidity of the venous blood.

Secretory activity occurring along with decreased blood supply was accompanied by a greater increase in blood acidity.

Secretion with the duct occluded caused greater than normal increase in blood acidity.

Increased acidity of the blood produced by mechanical asphyxia resulted in decreased resistance of the gland.

No generalization relating resistance or acidity changes to electrical deflections seems permissible at present.

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